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Cotinine assessed smoking status and chronic kidney disease of unknown origin in Guatemala

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Abstract

Objectives: In this paper we provide evidence that smoking exposure should be objectively measured with biomarkers rather than self-reported epidemiologic studies focused on chronic kidney disease of unknown origin (CKDu). Currently, there is a lack of information on cotinine levels in rural populations in low- and middle-income countries (LMICs) like Guatemala.

Design: We cross-sectionally evaluated self-reported smoking status against urinary cotinine concentrations, the gold standard biomarker of tobacco smoke exposure, among agricultural workers at four separate time points.

Setting: Guatemala.

Participants: 283 sugarcane workers.

Primary outcome measures: Compared self-reported smoking status and urinary cotinine concentrations in two agricultural worker studies.

Results: Self-reported smoking prevalence was 12% among workers. According to cotinine concentrations (≥ 50 ng/mL), the smoking prevalence was 34%. Self-reported smoking status had 28% sensitivity and 96% specificity. Urinary cotinine concentrations show that smoking prevalence is underestimated in this worker population.

Conclusions: Self-reported smoking status is likely an underestimate of the true smoking prevalence among agricultural workers. Research on the CKDu epidemic in Central America and other parts of the world might be underestimating tobacco exposure as a potential contributor to the development of disease. This report reinforces the need to further explore smoking status and biomarkers of tobacco use in epidemiologic research in rural, low-income populations, in particular those at-risk for CKDu.

Keywords: kidney disease, smoking, cotinine, public health

Strengths and limitations of this study:

- In this study we evaluated self-reported smoking status as measured against urinary cotinine levels among rural, agricultural workers.
- Results reinforce that misclassification of smoking status likely occurs due to the self-reported nature of the exposure.
- This misclassification could potentially be leading to the underestimation of the harmful effects of smoking on populations at-risk for chronic kidney disease of unknown origin (CKDu).
- A limitation is that there may be overlap between cotinine concentrations of non-smokers exposed to high levels of secondhand smoke and light/non-daily smokers.

Word count: 2124

Introduction

Smoking as a potential contributor to the development of Chronic Kidney Disease of Unknown Origin (CKDu) has not been fully explored and is often overlooked in CKDu research. The emerging CKDu epidemic has been documented over the past two decades throughout Latin America, Sri Lanka, and India.(1) CKDu is not related to established and typical CKD risk factors such as diabetes or hypertension and the etiology remains a mystery.(1) Several risk factors for the development of CKDu have been proposed, such as heat stress, dehydration, environmental exposures, infectious agents, medications, as well as a multifactorial etiology.(1-6)

Smoking, as opposed to other chronic kidney disease (CKD) risk factors, has received less attention in epidemiologic studies in populations at-risk for CKDu despite the evidence of its role with CKD. Scientific literature provides both mechanistic and epidemiologic evidence linking smoking to kidney disease and it is an established and independent CKD risk factor.(7-9) While few studies have found an association between self-reported smoking and CKDu (10-13), there may be multiple reasons for the lack of association findings. First, tobacco use misclassification is common and there is considerable heterogeneity between misclassification rates (14), especially among intermittent smokers.(15) This misclassification potentially leads to the underestimation of the harmful effects of smoking. Here we present data on the validity of self-reported smoking status as measured against urinary cotinine levels in a sample of workers at-risk for CKDu in Guatemala. Second, research on how smoking is assessed in rural populations in low-income countries where CKDu is endemic is lacking. Smoking patterns in these CKDu endemic areas may be different from those in urban populations and in high-income countries, where smoking is intermittent.(16-19) Third, while smoking may not be the main cause of CKDu, it may serve as an effect modifier or accelerate disease progression, as it has been well established risk factor for the development of CKD.(7)

In this report, we provide evidence that smoking should be objectively measured using biomarkers, such as cotinine, in epidemiological studies of CKDu and should not be assessed in the same manner as in high-income countries where smoking practices are likely different.

Methods

a. Study Design

The data for this analysis were derived from two studies among male agricultural workers (≥ 18 years) employed by a sugarcane agribusiness in Guatemala. One study was conducted during the 2017-2018 harvest among 202 field workers and the other study during the 2016-2017 harvest among 81 field workers. The harvest season lasts 6 months from November through May.

The 2017-2018 participants were randomly recruited within four work groups of cane cutters in November 2017. The 2016-2017 participants were randomly recruited within a similar but separate population of workers in December 2016. During the 2017-2018 study, we collected survey data and spot urine samples at three time points: November 2017 (4 groups), January 2018 (2 randomly selected groups among the 4 groups), and April 2018 (4 groups). During the 2016-2017 study, we collected survey data and urine samples in February 2017. This gave us a total of 283 matched urine and participant surveys with self-reported smoking status.

At enrollment in November 2017, participants were asked “Do you smoke cigarettes?” by a Spanish-speaking interviewer (not employed by the agribusiness). Participants responded that they were either current smokers, former smokers, or had never smoked. Former smokers were also asked “How old were you when you quit smoking?”. In January and April 2018, the participants were asked at the end of their 8-10-hour work shift “How many cigarettes have you smoked since you woke up this morning?”. This question was also asked at the end of the work shift in February 2017 during the 2016-2017 study. All urine samples were collected in morning except at the November timepoint, where 95 urine samples (47%) were collected in the afternoon. A common practice with urine analytes is to

correct for urine creatinine to adjust for dilutional effects, however studies documenting the usefulness of correcting cotinine concentrations for urine creatinine are limited and may not be necessary. (20) We did not to correct for urine creatinine based on the limited correction information and after establishing that our afternoon urine creatinine levels were dilute, and dehydration was not a concern.

All participants provided written consent prior to enrollment. Both protocols were approved by the Institutional Review Board of the University of Colorado (COMIRB, #16-1824 and #17-1328) and in Guatemala by the Comité de Ética Independiente ZUGUEME (2017-18 study) and by the Comité de Ética, Facultad de Medicina, Universidad Francisco Marroquin-Hospital Universitario Esperanza (2016-17 study). Workers were involved in the design and implementation plans of this research, but they were not involved in the data collection, analysis, or interpretation of this research.

b. Laboratory Analysis

Urine cotinine levels were determined using the Calbiotech Cotinine ELISA CO096D (Calbiotech, El Cajon, California) and the limit of detection (LOD) was 5 ng/mL. A cotinine-verified non-smoker was defined as having urinary cotinine ≤ 50 ng/mL. This threshold was used as the cut-off according to the Society for Research on Nicotine and Tobacco and is consistent with being a current smoker. (21)

c. Statistical Analysis

We compared self-reported smoking status and urine cotinine categories (> 50 vs. ≤ 50 ng/mL) in November 2017. Participants were excluded (n=50) if they had missing survey data or urine samples. Agreement between self-report and cotinine concentrations was assessed using the McNemar's test. We calculated sensitivity (those with cotinine > 50 ng/mL and reported being a current smoker) and specificity (those with cotinine ≤ 50 ng/mL and reported being a nonsmoker) by using urinary measurements as the gold standard. Similarly, at the three other time points (January and April 2018; February 2017), cotinine categories were compared to self-reported cigarette use on the study day. All data analyses were performed in SAS version 9.4 (SAS Institute, Inc., Cary, NC).

Results

November 2017 urinary cotinine concentration distributions for the 202 participants are presented in Table 1 and Figure 1. Among the 150 participants (74%) who reported had never smoked, 39 participants (26%) had cotinine levels > 50 ng/mL and among the 28 participants (14%) who reported being a former smoker, 10 participants (36%) had cotinine levels > 50 ng/mL. Based on responses to the question "How old were you when you quit smoking?", none of the former smokers had high cotinine concentrations due to having just quit. Self-reported smoking status had a sensitivity of 28% and specificity of 96%. Smoking status and cotinine levels were not associated (McNemar's test, $p<0.05$).

For the 2017-2018 and 2016-2017 studies, the percent of participants who reported no cigarettes the day of the study but had cotinine levels > 50 ng/mL was 26% in January, 25% in April, and 21% in February 2017 (Table 2).

Therefore, using these two populations of agricultural field workers, we found that approximately 25% of participants who would be considered a non-smoker based on self-reported smoking status, had an objective measurement of recent tobacco exposure with a urine cotinine concentration > 50 ng/mL.

Discussion

In this study, we evaluated self-reported smoking status of agricultural workers using an established biomarker of smoke exposure, urinary cotinine. We observed that self-reported smoking status likely underestimates smoking prevalence in this population. Twelve percent of the participants reported being a current smoker, however, 34% of participants had cotinine levels that would classify them as current smokers. The self-reported smoking prevalence in our study was much lower than the last national survey on smoking in Guatemala (2003) where 24% of males self-reported as current smokers (definition: smoked ≥ 1 cigarettes in the past 30 days).(22)

Data on smoking exposure in CKDu studies have mostly been dependent on self-reports and have yielded conflicting findings on the association of smoking and kidney dysfunction. Several studies have found that current or ever smokers are at a higher risk of kidney dysfunction. Among 330 sugarcane workers in Guatemala, we found that self-reported current smokers (vs. never or former smokers) were at a significantly greater risk for a decline in kidney function over a harvest season.(10) Two studies conducted in Sri Lankan agricultural workers found that smoking (current or ever) was found to be a risk factor for CKDu.(12, 13) In addition, patients with biopsy-proven tubulointerstitial kidney disease in Sri Lanka were more likely to have ever used tobacco.(11) Several studies in Nicaragua have found smoking to be a risk factor in univariate analyses and smoking was either controlled for in the multivariable analysis or was no longer significant.(23-25) Other studies found no relationships between smoking and kidney dysfunction and/or very low prevalence numbers.(26-30) There was a wide prevalence range of self-reported smoking among these community and worker studies. While smoking assessments varied substantially, the most common question to assess smoking was whether participants were current or ever smokers.

Tobacco use misclassification could introduce bias and be one reason for these conflicting results; true smoking rates are likely underestimated as our current findings yield. This misclassification bias could potentially be leading to the underestimation of the harmful effects of smoking on populations at-risk for CKDu. In addition, rates of smoking misclassification have been found to be higher in diseased groups and case-control studies, suggesting that presence of disease may affect smoking status response. (14)

Smoking status misclassification is likely due to several reasons. One is the social desirability bias, where smokers misclassify themselves as non-smokers due to cultural pressure to quit smoking. In addition, these data were collected at a worksite with a smoke-free workplace policy in place. Although workers were assured that their survey responses would be kept confidential, workers may have felt

pressure to deny smoking. Another reason is the difference in smoking patterns between high-income countries and low- and middle-income countries (LMIC) where CKDu is endemic. One study found differing patterns of current smoking and type of cigarette smoked (light vs. regular) across Brazil, China, Mexico, and Poland. (31) In Guatemala, like other Latin American countries and Latinos in the United States, light smoking and non-daily smoking is highly prevalent.(18, 19) Furthermore, single-cigarette sales are very common in Guatemala City and neighboring towns.(16) In another study conducted from 2001-2004, it was found that non-daily smoking was common among men in several Central American countries (42% in El Salvador, 23% in Guatemala and 19% in Honduras).(18) These studies provide insight on smoking patterns in CKDu endemic countries in Central America; it is very possible that light or non-daily smokers feel as if they are not true smokers and thus self-report as non-smokers in epidemiologic surveys. Capturing different smoking patterns among populations at-risk for CKDu is an essential step toward accurately documenting tobacco smoke exposure in epidemiologic research. While survey-based, self-reported smoking is commonly used to assess smoking status due to its low cost, and ease of use, investigators should be cautious when interpreting smoking prevalence given our findings that self-reported smoking status can be inaccurate. Regular validity tests (i.e. biomarkers of smoke exposure) should be performed to compensate for the limitations of self-reported smoking surveys. In addition, questions should aim to capture patterns of both daily smokers and less than daily smokers. A 2014 Global Adult Tobacco Survey (GATS) stressed the importance of three basic questions to measure tobacco smoking prevalence, which includes questions on current use (both daily and less than daily responses available), past daily use less than daily smokers, and past use for current non-smokers. These type of improved survey questions on smoking exposure should be evaluated with objective measure, such as cotinine in epidemiological studies of CKDu.

Our study findings have some limitations. It may be difficult to generalize the findings of our study because findings are based on a rural agricultural worker population. While cotinine is commonly

used to discern smokers from nonsmokers, there may be overlap between cotinine concentrations of non-smokers exposed to high levels of secondhand smoke and light/non-daily smokers. Further research should focus on assessing the optimal cut-off point for validating smoking status among agricultural populations in LMIC. While the discrepancy between urinary cotinine concentrations and cigarette use on the study day is consistent between January and April 2018 and February 2017, we must interpret these findings with caution. A cotinine concentration > 50 ng/mL might reflect a current smoker who did not smoke any cigarettes on the study day and could reflect smoking the previous day. In addition, 24-hour urine sample collection would be a more reliable parameter for the assessment of diuresis, although less feasible in epidemiologic studies.

In this report we are not taking the position that smoking is the sole cause of CKDu. However cigarette smoking is a well-established and important modifiable risk factor for several diseases, including CKD.(7) We have a disease of "unknown" origin and tobacco use has not been fully explored, in part due to self-report misclassification. Thus, understanding unique aspects of tobacco smoking is needed among populations at-risk for CKDu and future studies need more objective measurements of smoking as a risk factor for the development of CKDu.

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Conflict of Interest: University of Colorado and Pantaleon are separate, independent organizations. University of Colorado employed appropriate research methods in keeping with academic freedom, based conclusions on critical analysis of the evidence and reported findings fully and objectively. The terms of this arrangement have been reviewed and approved by the University of Colorado in accordance with its conflict of interest policies.

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Author Contributions: Conceptualization: JBD, JB. Data curation: LK. Formal analysis: WF, JBD. Funding acquisition: LN. Methodology: JBD, SB, LK, LN. Project administration: LK. Visualization: JBD, JB, LN. Writing - original draft: JBD, SB. Writing - review & editing: JB, LK, SB, WF, CA, LN.

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Figure Legend

Figure 1: Box plots of log (10) urine cotinine concentrations by self-reported smoking status in November.

Table 1: Urine cotinine concentrations by self-reported smoking status in November 2017, N=202

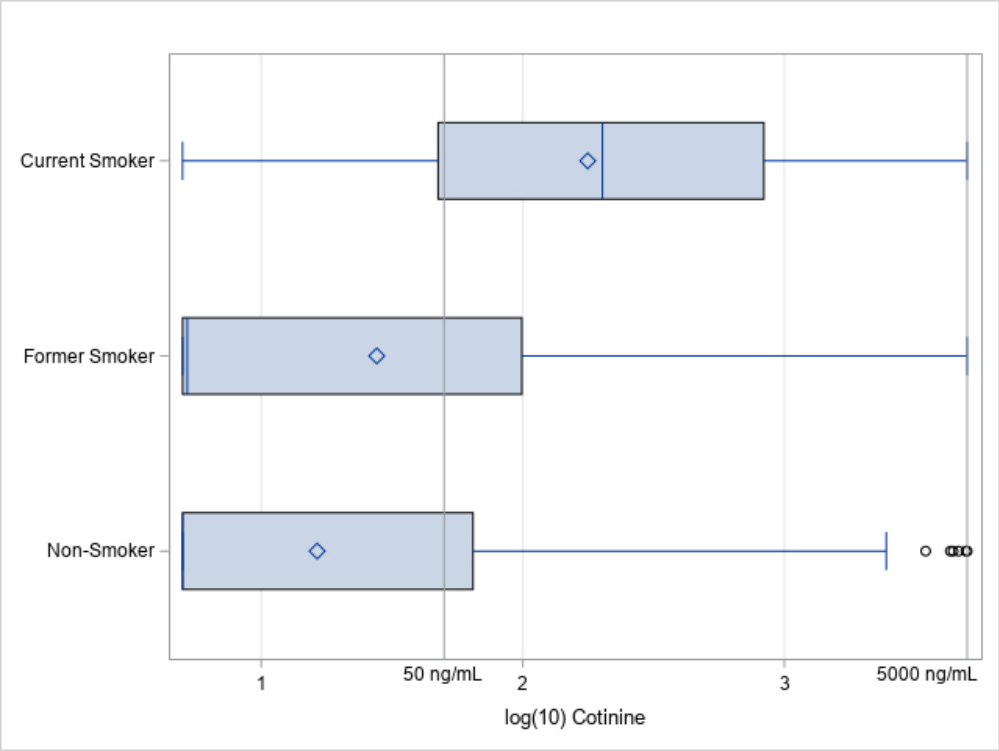
Self-reported smoking status[1]	Age, mean (SD)	N (%)	Cotinine Concentrations (ng/mL)[2]				
			Median (IQR)	p-value[A]	Cotinine, ≤ 50, N (%)	Cotinine, >50, N (%)	p-value[3]
All participants	29 (8)	202 (100%)	<LOD (<LOD, 106.75)		134 (66%)	68 (34%)	
Current Smoker	30 (8)	24 (12%)	310.03 (76.23, 650.58)	<0.01	5 (21%)	19 (79%)	<0.01
Former Smoker	32 (9)	28 (14%)	7.84 (<LOD, 92.98)		18 (64%)	10 (36%)	
Never smoked	28 (8)	150 (74%)	<LOD (<LOD, 63.34)		111 (74%)	39 (26%)	

Abbreviations: SD, standard deviation; IQR, interquartile range.
[1] November survey question: "Do you smoke cigarettes?"
[2] Values above 5000 ng/mL were put at 5000 ng/mL. Limit of detection = 5 ng/mL.
[3] P value for overall difference between smoking groups.

Table 2: Urinary cotinine concentrations (≤ 50 vs. > 50 ng/mL) by reported cigarette use on study day, n (%).

2017-2018 Study[1]			
January, n=92	**Overall**	**≤ 50 ng/mL**	**>50 ng/mL**
Smoked Cigarette(s)	1 (1%)	0 (0%)	1 (100%)
Did not smoke	91 (99%)	67 (74%)	24 (26%)
April, n=167	**Overall**	**≤ 50 ng/mL**	**>50 ng/mL**
Smoked Cigarette(s)	10 (6%)	2 (20%)	8 (80%)
Did not smoke	157 (94%)	117 (75%)	40 (25%)
2016-2017 Study			
February, n=81	**Overall**	**≤ 50 ng/mL**	**>50 ng/mL**
Smoked Cigarette(s)	4 (5%)	2 (50%)	2 (50%)
Did not smoke	77 (95%)	61 (79%)	16 (21%)

[1] Survey question: "How many cigarettes have you smoked since you woke up this morning?"



STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	5-6
Study size	10	Explain how the study size was arrived at	5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	6
Outcome data	15*	Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A

		(*b*) Report category boundaries when continuous variables were categorized	Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Accuracy of self-reported smoking status as compared to urinary cotinine levels among workers at risk for chronic kidney disease of unknown origin in Guatemala.

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Accuracy of self-reported smoking status as compared to urinary cotinine levels among workers at risk for chronic kidney disease of unknown origin in Guatemala.

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Abstract

Objectives: There is a lack of information on cotinine levels in rural populations in low- and middle-income countries (LMICs) like Guatemala. Therefore, there is a need to explore smoking status and biomarkers of tobacco use in epidemiologic research in rural, low-income populations, in particular those at-risk for chronic kidney disease of unknown origin (CKDu).

Design: We evaluated self-reported smoking status against urinary cotinine levels, the gold standard biomarker of tobacco smoke exposure, among agricultural workers at four separate cross-sectional time points.

Setting: Guatemala.

Participants: 283 sugarcane workers.

Primary outcome measures: Compared self-reported smoking status and urinary cotinine levels in two agricultural worker studies.

Results: Self-reported smoking prevalence was 12% among workers. According to cotinine levels (≥ 50 ng/mL), the smoking prevalence was 34%. Self-reported smoking status had 28% sensitivity and 96% specificity. Urinary cotinine levels show that smoking prevalence is underestimated in this worker population.

Conclusions: According to our findings, smoking status should be objectively measured with biomarkers rather than self-reported in CKDu epidemiologic research. Self-reported smoking status is likely an underestimate of the true smoking prevalence among agricultural workers. Research on the CKDu epidemic in Central America and other parts of the world might be underestimating tobacco exposure as a potential contributor to the development of CKDu.

Keywords: kidney disease, smoking, cotinine, public health

Strengths and limitations of this study:

- The study provides an international view of the importance of adequately assessing smoking prevalence by validating the accuracy of self-reported smoking questionnaires.
- The misclassification bias of smokers needs to be examined in rural populations.
- Urine cotinine and self-reported smoking status were investigated concurrently at multiple cross-sectional time points.
- The study results may have limited generalizability as it was conducted among agricultural workers in Guatemala.

Word count: 2244

Introduction

Smoking as a potential contributor to the development of Chronic Kidney Disease of unknown origin (CKDu) has not been fully explored and is often overlooked in CKDu research. The emerging CKDu epidemic has been documented over the past two decades throughout Latin America, Sri Lanka, and India.(1) CKDu is not associated with established chronic kidney disease (CKD) risk factors such as diabetes or hypertension and the etiology remains unknown.(1) Several CKDu risk factors have been proposed, including heat stress, dehydration, environmental exposures, infectious agents, medications, as well as a multifactorial etiology.(1-6)

Smoking, as opposed to other CKD risk factors, has received less attention in epidemiologic studies in populations at-risk for CKDu despite the evidence of its role with CKD. Scientific literature provides both mechanistic and epidemiologic evidence linking smoking to kidney disease and it is an established and independent CKD risk factor.(7-9) While few studies have found an association between self-reported smoking and CKDu (10-13), there may be multiple reasons for the lack of association findings. First, tobacco use misclassification is common and there is considerable heterogeneity between misclassification rates (14), especially among light or non-daily smokers.(15) This misclassification potentially leads to the underestimation of the harmful effects of smoking. Here we present data on the validity of self-reported smoking status against urinary cotinine levels in a sample of workers at-risk for CKDu in Guatemala. Cotinine, the main nicotine metabolite, accumulates in the body as a result of tobacco exposure and can be easily detected in urine, blood, and saliva. Urine cotinine is commonly used as an objective measure to distinguish tobacco users and non-users.(16) Second, research on how smoking is assessed in rural populations in low-income countries where CKDu is endemic is lacking. Smoking patterns in CKDu endemic areas may be different from those in urban populations and in high-income countries, where smoking is intermittent.(17-20) Third, while smoking

may not be the main cause of CKDu, it may serve as an effect modifier or accelerate disease progression, as it is a well-established CKD risk factor.(7)

Therefore, we provide evidence that smoking should be objectively measured using biomarkers, such as cotinine, in CKDu epidemiological studies and should not be assessed in the same manner as in high-income countries where smoking practices are likely different.

Methods

a. Study Design

The data for this analysis were derived from two studies among male agricultural workers (≥ 18 years) employed by a sugarcane agribusiness in Guatemala. One study was conducted during the 2016-2017 harvest among 81 field workers and the other study during the 2017-2018 harvest among 202 field workers. The harvest season lasts 6 months from November through May.

The 2016-2017 participants were randomly recruited within a population of workers in December 2016. The 2017-2018 participants were a similar but separate population of workers and were randomly recruited within four work groups of workers in November 2017. During the 2016-2017 study, we collected survey data and spot urine samples in February 2017. During the 2017-2018 study, we collected survey data and spot urine samples at three time points: November 2017 (4 groups), January 2018 (2 randomly selected groups among the 4 groups), and April 2018 (4 groups). This gave us a total of 283 matched urine and participant surveys with self-reported smoking status.

For the 2016-2017 study, participants were asked at the end of their 8-10-hour work shift in February “How many cigarettes have you smoked since you woke up this morning?” by a Spanish-speaking interviewer (not employed by the agribusiness). At enrollment in November for the 2017-2018 study, participants were asked “Do you smoke cigarettes?”. Participants responded that they were either current smokers, former smokers, or had never smoked. Former smokers were also asked “How old were you when you quit smoking?”. At the other two time points during the 2017-2018 study,

January and April, the participants were asked at the end of their 8-10-hour work shift "How many cigarettes have you smoked since you woke up this morning?".

Urine samples were collected in morning except at the November timepoint, where 95 samples (47%) were collected in the afternoon. A common practice with urine analytes is to correct for urine creatinine to adjust for dilutional effects. However, studies documenting the usefulness of correcting cotinine levels for urine creatinine are limited and may not be necessary.(21) We did not to correct for urine creatinine based on the limited correction information and after establishing that our afternoon urine creatinine levels were dilute, and dehydration was not a concern.

All participants provided written consent prior to enrollment. Both protocols were approved by the Institutional Review Board of the University of Colorado (COMIRB, #16-1824 and #17-1328) and in Guatemala by the Comité de Ética, Facultad de Medicina, Universidad Francisco Marroquin-Hospital Universitario Esperanza (2016-17 study) and the Comité de Ética Independiente ZUGUEME (2017-18 study).

b. Laboratory Analysis

Urine cotinine levels were determined using the Calbiotech Cotinine ELISA CO096D (Calbiotech, El Cajon, California) and the limit of detection (LOD) was 5 ng/mL. A cotinine-verified non-smoker was defined as having urinary cotinine ≤ 50 ng/mL. This threshold was used as the cut-off according to the Society for Research on Nicotine and Tobacco and is consistent with being a current smoker.(22)

c. Statistical Analysis

We compared self-reported smoking status and urine cotinine categories (≤ 50 vs. > 50 ng/mL) in November 2017. Participants were excluded (n=50) if they had missing survey data or urine samples. Agreement between self-report and cotinine levels was assessed using the McNemar's test. We calculated sensitivity (cotinine > 50 ng/mL and reported being a current smoker) and specificity (cotinine ≤ 50 ng/mL and reported being a non-smoker) using urinary measurements as the gold standard.

Similarly, at the three other time points (February 2017, January and April 2018), cotinine categories were compared to self-reported cigarette use on the study day. All data analyses were performed in SAS version 9.4 (SAS Institute, Inc., Cary, NC).

d. Patient and Public Involvement

These two studies included a collaborative process that engaged workers and worker representatives in the development, implementation, and dissemination plans of the research to enhance its relevance and impact. They were not involved in the data analysis or interpretation of this research.

Results

November 2017 urinary cotinine level distributions for the 202 participants are presented in Table 1 and Figure 1. Among the 150 participants (74%) who reported had never smoked, 39 (26%) had cotinine levels > 50 ng/mL and among the 28 (14%) who reported being a former smoker, 10 (36%) had cotinine levels > 50 ng/mL. Based on responses to the question "How old were you when you quit smoking?", none of the former smokers had high cotinine levels due to having just quit. To assess the accuracy of self-reported data, sensitivity and specificity were calculated. Self-reported smoking status had a sensitivity of 28% and specificity of 96%, indicating that 72% of the workers identified as smokers by the urine cotinine test reported being a former or never smoker and 4% of workers identified as non-smokers by the urine cotinine test reported themselves as a current smoker. Smoking status and cotinine levels were not associated (McNemar's test, $p<0.05$).

For both the studies, the percent of participants who reported no cigarettes the day of the study but had cotinine levels > 50 ng/mL was 21% in February 2017, 26% in January 2018, and 25% in April 2018 (Table 2).

Therefore, using these two study populations of agricultural field workers, we found that approximately 25% of participants who would be considered a non-smoker based on self-reported

smoking status, had an objective measurement of recent tobacco exposure with urine cotinine concentration > 50 ng/mL.

Discussion

In this study, we evaluated self-reported smoking status of agricultural workers using an established biomarker of smoke exposure, urinary cotinine. We observed that self-reported smoking status likely underestimates smoking prevalence in this population. The prevalence of smoking was 34% based on cotinine and 12% based on self-reported data, indicating that self-reporting led to an underestimation of smoking by 64%. The self-reported smoking prevalence in our study was much lower than the last national survey on smoking in Guatemala (2003) where 24% of males self-reported as current smokers (definition: smoked ≥ 1 cigarettes in the past 30 days).(23, 24)

Data on smoking exposure in CKDu studies have mostly been dependent on self-reports and yielded conflicting findings on the association with kidney dysfunction. Several studies have found that current or ever smokers are at a higher risk of kidney dysfunction. Among 330 sugarcane workers in Guatemala, we found that self-reported current smokers (vs. never or former smokers) were at a significantly greater risk for a decline in kidney function over a harvest season.(10) Two studies conducted in Sri Lankan agricultural workers found that smoking (current or ever) was a risk factor for CKDu.(12, 13) In addition, patients with biopsy-proven tubulointerstitial kidney disease in Sri Lanka were more likely to have ever used tobacco.(11) Three studies in Nicaragua have found smoking to be a risk factor in univariate analyses and smoking was either controlled for in the multivariable analysis or was no longer significant.(25-27) Other studies found no relationships between smoking and kidney dysfunction and/or very low smoking prevalence .(28-32) There was a wide prevalence range of self-reported smoking among these community and worker studies. While smoking assessments varied substantially, the most common question to assess smoking was whether participants were current or ever smokers.

Tobacco use misclassification could introduce bias and be one reason for these conflicting results; true smoking rates are likely underestimated as our current findings yield. This misclassification bias could potentially be leading to the underestimation of the harmful effects of smoking on populations at-risk for CKDu. In addition, rates of smoking misclassification have been found to be higher in diseased groups and case-control studies, suggesting that presence of disease may affect smoking status response. (14)

Smoking status misclassification is likely due to several reasons. One is the social desirability bias, where smokers misclassify themselves as non-smokers due to cultural pressure to quit smoking. In addition, these data were collected at a worksite with a smoke-free workplace policy in place. Although workers were assured that their survey responses would be kept confidential, they may have felt pressure to deny smoking. Another reason is the difference in smoking patterns between high-income countries and low- and middle-income countries (LMIC) where CKDu is endemic. One study found differing patterns of current smoking and type of cigarette smoked (light vs. regular) across Brazil, China, Mexico, and Poland. (33) In Guatemala, like other Latin American countries and Latinos in the United States, light smoking and non-daily smoking is highly prevalent.(19, 20) Furthermore, single-cigarette sales are very common in Guatemala City and neighboring towns.(17) In another study conducted between 2001-2004, it was found that non-daily smoking was common among men in several Central American countries (42% in El Salvador, 23% in Guatemala and 19% in Honduras).(19) These studies provide insight on smoking patterns in CKDu endemic countries in Central America; it is very possible that light or non-daily smokers do not consider themselves "smokers" and may under-report their cigarette use in epidemiologic surveys.(34, 35) Capturing different smoking patterns among populations at-risk for CKDu is an essential step toward accurately documenting tobacco smoke exposure in epidemiologic research. While survey-based, self-reported smoking is commonly used to assess smoking status due to its low cost, and ease of use, investigators should be cautious when interpreting smoking

prevalence given our findings that self-reported smoking status can be inaccurate. Regular validity tests (i.e. biomarkers of smoke exposure) should be performed to compensate for the limitations of self-reported smoking surveys. In addition, questions should aim to capture patterns of both daily and non-daily smokers. A 2014 Global Adult Tobacco Survey (GATS) stressed the importance of three basic questions to measure tobacco smoking prevalence, which includes questions on current use (both daily and less than daily responses available), past use for non-daily smokers, and past use for current non-smokers. These type of improved survey questions on smoking exposure should be evaluated with objective measure, such as cotinine in epidemiological studies of CKDu.

Our study findings have some limitations. It may be difficult to generalize the results as they are from a rural agricultural worker population. While cotinine is commonly used to discern smokers from nonsmokers, there may be overlap between cotinine levels of non-smokers exposed to high levels of secondhand smoke and light/non-daily smokers. Further research should focus on assessing the optimal cut-off point for validating smoking status among agricultural populations in LMIC. While the discrepancy between urinary cotinine levels and cigarette use on the study day is consistent between February 2017, January 2018, and April 2018, we must interpret these findings with caution. A cotinine level > 50 ng/mL might reflect a current smoker who did not smoke any cigarettes on the study day and could reflect smoking the previous day. In addition, 24-hour urine sample collection would be a more reliable parameter for the assessment of diuresis, although less feasible in epidemiologic studies.

In this report we are not taking the position that smoking is the sole cause of CKDu. However smoking is a well-established and important modifiable risk factor for several diseases, including CKD.(7) We have a disease of “unknown” origin and tobacco use has not been fully explored, in part due to self-report misclassification. Thus, understanding unique aspects of smoking is needed among populations at-risk for CKDu and future studies need more objective measurements of smoking as a risk factor for the development of CKDu.

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Conflict of Interest: University of Colorado and Pantaleon are separate, independent organizations. University of Colorado employed appropriate research methods in keeping with academic freedom, based conclusions on critical analysis of the evidence and reported findings fully and objectively. The terms of this arrangement have been reviewed and approved by the University of Colorado in accordance with its conflict of interest policies.

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Data Sharing Agreement: The data that support the findings of this study are available from the corresponding author, JBD, upon reasonable request.

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Figure Legend

Figure 1: Box plots of log (10) urine cotinine levels by self-reported smoking status in November 2017.

Table 1: Urine cotinine levels by self-reported smoking status in November 2017, N=202.

Self-reported smoking status[1]	Age, mean (SD)	N (%)	Cotinine Levels (ng/mL)[2]				
			Median (IQR)	p-value[3]	Cotinine, > 50, N (%)	Cotinine, ≤ 50, N (%)	p-value[3]
All participants	29 (8)	202 (100%)	<LOD (<LOD, 106.75)		68 (34%)	134 (66%)	
Current Smoker	30 (8)	24 (12%)	310.03 (76.23, 650.58)	<0.01	19 (79%)	5 (21%)	<0.01
Former Smoker	32 (9)	28 (14%)	7.84 (<LOD, 92.98)		10 (36%)	18 (64%)	
Never smoked	28 (8)	150 (74%)	<LOD (<LOD, 63.34)		39 (26%)	111 (74%)	

Abbreviations: SD, standard deviation; IQR, interquartile range.

[1] November survey question: "Do you smoke cigarettes?"

[2] Values above 5000 ng/mL were put at 5000 ng/mL. Limit of detection = 5 ng/mL.

[3] P value for overall difference between smoking groups.

Sensitivity = 19 true positive smokers/ (19 + 10 + 39) * 100=28%.

Specificity = 129 true negative former and never smokers / (5 + 18 + 111) * 100=96%.

Table 2: Urinary cotinine levels (≤ 50 vs. > 50 ng/mL) by reported cigarette use on study day, n (%).

2016-2017 Study[1]			
February 2017, n=81	**Overall**	**≤ 50 ng/mL**	**>50 ng/mL**
Smoked Cigarette(s)	4 (5%)	2 (50%)	2 (50%)
Did not smoke	77 (95%)	61 (79%)	16 (21%)
2017-2018 Study[1]			
January 2018, n=92	**Overall**	**≤ 50 ng/mL**	**>50 ng/mL**
Smoked Cigarette(s)	1 (1%)	0 (0%)	1 (100%)
Did not smoke	91 (99%)	67 (74%)	24 (26%)
April 2018, n=167	**Overall**	**≤ 50 ng/mL**	**>50 ng/mL**
Smoked Cigarette(s)	10 (6%)	2 (20%)	8 (80%)
Did not smoke	157 (94%)	117 (75%)	40 (25%)

[1] Survey question: "How many cigarettes have you smoked since you woke up this morning?"

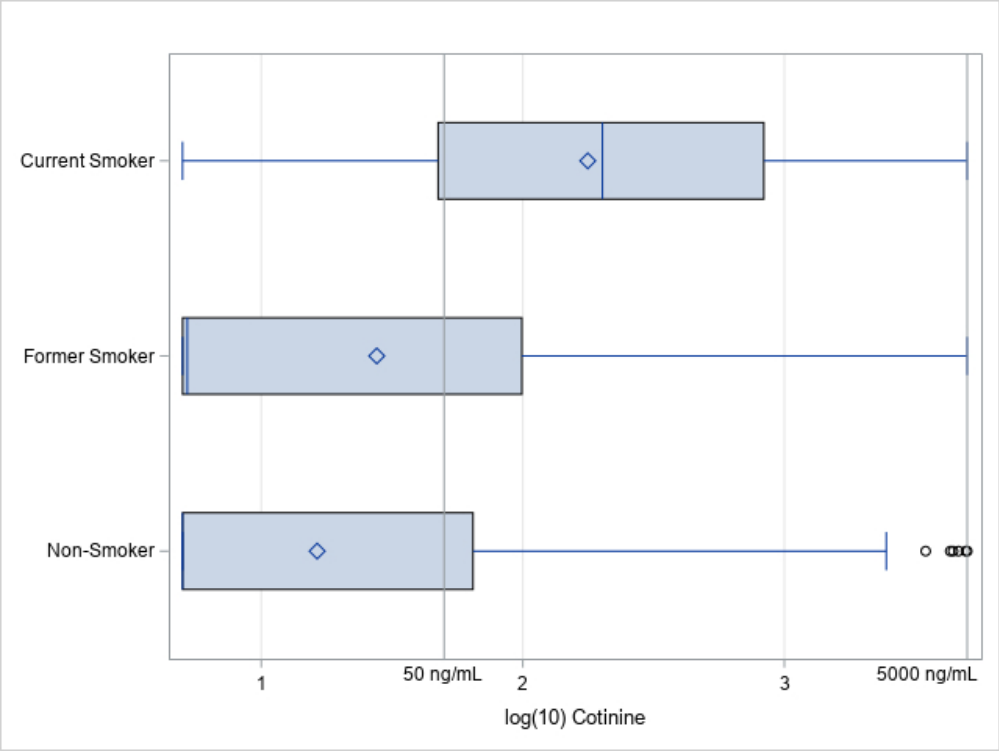

Figure 1: Box plots of log (10) urine cotinine levels by self-reported smoking status in November 2017.

427x320mm (38 x 38 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	5-6
Study size	10	Explain how the study size was arrived at	5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	6
Outcome data	15*	Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A

		(*b*) Report category boundaries when continuous variables were categorized	Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.